

Dietary supplementation with laminarin, a fermentable marine β (1–3) glucan, protects against hepatotoxicity induced by LPS in rat by modulating immune response in the hepatic tissue

Audrey M. Neyrinck, Ariane Mouson, Nathalie M. Delzenne*

Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, School of Pharmacy, Université catholique de Louvain, Brussels, Belgium

Received 12 February 2007; received in revised form 4 June 2007; accepted 24 June 2007

Abstract

We tested the hypothesis that laminarin (LAM), a β (1–3) polysaccharide extracted from brown algae, can modulate the response to a systemic inflammation. Male Wistar rats ($n=7$ per group) were fed a standard diet (control) or a diet supplemented with LAM for 25 days (5% during 4 days followed by 10% during 21 days). Thereafter, *Escherichia coli* lipopolysaccharides (LPS; 10 mg/kg i.p.) were injected and the animals were sacrificed 24 h after LPS challenge. The hypothermia, hyperglycemia and hypertriglyceridemia occurring early after LPS administration were less pronounced in LAM-treated rats than in controls. The increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities – reflecting hepatic alterations – was lessened after LPS injection in LAM-treated rats compared to control rats. LAM treatment decreased serum monocytes number, nitrite (NO_2) and tumor necrosis factor- α (TNF- α). LAM also modulated intra-hepatic immune cells: it lowered the occurrence of peroxidase-positive cells (corresponding to monocytes/neutrophils) and, in contrast, it increased the number of ED2-positive cells, corresponding to resident hepatic macrophages, *i.e.* Kupffer cells. In conclusion, the hepatoprotective effect of marine β (1–3) glucan during endotoxic shock may be linked to its immunomodulatory properties. We propose that both lower recruitment of inflammatory cells inside the liver tissue and lower secretion of inflammatory mediators play a role in the tissue protective effect of LAM. These effects could be due to a direct effect of β -glucan on immune cells, or to an indirect effect through their dietary fibre properties (fermentation in the gut).

© 2007 Elsevier B.V. All rights reserved.

Keywords: Kupffer cells; β -glucan; Laminarin; LPS

1. Introduction

Systemic inflammatory syndrome represents a major cause of morbidity and mortality in medical facilities today. High incidence of systemic infection by bacteria is probably related to several key factors. First, increased bacterial virulence and drug resistance have complicated treatment and led to problems in tracking disease [1,2]. Second, the host defence capacity of many

Abbreviations: ALT, Alanine aminotransferases; AST, Aspartate aminotransferases; FOS, fructo-oligosaccharides; GIT, gastro-intestinal tract; LAM, laminarin; LDH, Lactate dehydrogenase; LPS, lipopolysaccharides.

* Corresponding author. PMNT 7369, Université catholique de Louvain, 73 avenue Mounier, B-1200 Brussels, Belgium. Tel.: +32 2 764 73 39; fax: +32 2 764 73 59.

E-mail address: Nathalie.delzenne@uclouvain.be (N.M. Delzenne).

patients has been compromised by the increased of immunosuppressive therapies. Finally, the incidence of opportunistic infections has grown rapidly due to the worldwide AIDS epidemic [3,4]. To counteract the problems of drug resistance, there is a need to develop and characterize alternative anti-infective substances as adjuvants to classical antibiotic therapy [3]. One of the most promising recent alternatives is the use of immunomodulators for enhancing host defence responses. Modulation of immune function by nutrients is an emerging research area in the field of nutrition [5]. In a recent study, we have shown that non-digestible fructo-oligosaccharides (FOS) that are largely fermented by the microflora in the colon, when added in the diet (10%), protects the liver from injury induced by a single LPS injection in rats. This effect is namely due to the stimulation of hepatic immune cells [6]. The liver is directly linked to the gastro-intestinal tract (GIT) through the portal venous system. The high local blood flow results in a high rate of interaction of hepatic immune and sinusoidal cells with nutrients or nutrient metabolites, but also with foreign antigens. This characteristic confers to the liver a central immunologic function [7]. Events occurring in the GIT can thus have consequences on hepatic immune cells, including Kupffer cells. Kupffer cells, localised mainly in the periportal area in hepatic sinusoids, represent 80–90% of fixed macrophages of the whole body. Kupffer cells thus constitute the first macrophage population outside the gut coming into contact with bacteria, endotoxin (LPS) and microbial debris [8]. Consequently, Kupffer cells play a major role in clearing circulating LPS from the blood [9,10].

When Gram-negative bacteria, normally restricted to the GIT, invade the bloodstream, circulatory collapse, multiple organ failure and ultimately death can occur [11]. LPS, a component of the Gram-negative bacterial cell wall, is responsible for initiating a series of a highly complex cascading events leading to damage in multiple organs, including the liver and the lung. In a rat model of systemic inflammation induced by administration of high doses LPS (10 mg/kg), lesions were observed both in the liver and in the lung [12]. Several mediators are secreted by macrophages upon LPS stimulation [13,14]. Some of them are cytokines – TNF- α being the most important – which participate to multiple organ failure. The intravenous injection of TNF- α induces a shock syndrome similar to the one caused by LPS [11].

Our study focuses on gut–liver interactions to support the importance of interesting nutrients – β (1–3) glucans – in the control of systemic infection by Gram-negative bacteria. Seaweeds, approved for human

consumption, are rich in polysaccharides, in particular linear β (1–3) glucan, with β (1–6) side chains of varying length and distribution [15]. Most of them form complex structures stabilized by inter-chain hydrogen bonds and are thus resistant to hydrolysis in the upper gastro-intestinal tract: they can be considered as dietary fibres [16]. In the present study, we tested the hypothesis that LAM, a β (1–3) glucan extracted from brown algae, is fermented in the caeco-colon when given in the diet, and can, as shown previously for other fermentable carbohydrates, modulate the metabolic and toxic response to LPS administration by changing the pattern of immune cells present in the liver tissue. We have tested the putative implication of hepatic immune cells by analysing specific markers of Kupffer cells (ED2 membrane antigen) and myeloperoxidase-positive tissue cells; inflammatory mediators produced by hepatic cells, and that are clearly related to modulation of hepatotoxicity have been measured: the pro-inflammatory cytokine (TNF- α), a reactive nitrogen intermediate (NO₂ reflecting NO₂ production) and the anti-inflammatory prostaglandin E₂ (PGE₂) largely secreted by Kupffer cells [17,18]. Moreover, we have performed a histological examination of most organ and have determined the relative proportions of the different types of white blood cell.

2. Materials and methods

2.1. Chemicals

Most chemicals of the purest grade available were purchased from Sigma (St Louis, MO), Merck (Darmstadt, Germany) or Roche Diagnostics Belgium (Brussels, Belgium).

Laminarin was provided by Goëmar (St Malo, France); the compound is a poly β (1–3) glucan with some β (1–6) branchings. It is composed of a major M-series containing 20–30 glycosyl residues linked to a mannitol terminal residue and a minor G-series containing 22–28 glycosyl residues. Both series have a mean degree of polymerisation of 25 glycosyl residues, with an approximately M-series:G-series ratio of 3:1. The molecular weight was 4500 to 5500 g/mol and the purity was 90%. The nutritional composition of the compounds for 100 g was 1.3 g proteins, <0.1 g lipids and 91 g carbohydrates (energy value was 3.7 kcal/g or 15.7 kJ/g).

2.2. Animals and diet

Male Wistar rats ($n=7$ per group) weighing 136 ± 1 g (Harlan, Horst, The Netherlands) were housed in individual cages in a room with temperature control and an automatic 12 h light:dark cycle. After an acclimatation period of 5 days before the experiment, control rats (CT) were fed a powdered AO4 standard diet (UAR, Villemoisson-sur-Orge, France),

whereas laminarin treated-rats (LAM) received the same diet containing 5 g/100 g laminarin for 4 days followed by a dietary treatment of 10 g/100 g laminarin during 21 days. The AO4 standard diet contained the following (g/100 g dry diet): 19 protein; 70 total carbohydrates; 3.2 lipids; 7.3 minerals and vitamins [19]. Body weight as well as food and water intake were monitored two times per week. After 25 days of treatment, LPS from *Escherichia coli* 0127:B8 (10 mg/kg) were injected i.p. Food was withdrawn after LPS administration. Blood was collected from the tail vein 0 h, 2 h, 4 h, 6 h, 8 h after LPS injection and rats were killed under pentobarbital (Nembutal, 60 mg/kg body) anaesthesia 24 h after LPS administration. Blood was collected from the vena cava for white blood cell count or centrifuged to obtain serum which is stored at -80°C for further analysis. Liver, lung, caecum and spleen specimens were weighed, fixed in formalin and embedded in paraffin, and sections were stained with hematoxylin-eosin for histological analysis. Some liver pieces were stored at -80°C for further histological analysis. All rats received care in compliance with institution's guidelines from the National Academy of Sciences (NIH publication 86-23; <http://www.nih.gov>). The housing conditions were as specified by the Belgian Law of 14 November 1993 on the protection of laboratory animals (agreement no. LA 1230315). Experiments were approved by the Ethic Committee of the Université catholique de Louvain, Brussels, Belgium.

2.3. Rectal temperature

Rectal temperature was recorded using thermometer (Homeothermic Blanket Control Unit, Harvard™). All measurements were made at the temperature of $22\pm 2^{\circ}\text{C}$.

2.4. Biochemical parameters

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities as well as glucose and triglycerides were measured in frozen aliquots of serum by standardised enzymatic procedures using kits from Elitech (Sées, France).

2.5. Inflammatory mediators

PGE_2 and $\text{TNF-}\alpha$ concentrations were measured in frozen aliquots of serum by immunoassay kits (PGE_2 Immunoassay, DE0100 and Quantikine rat $\text{TNF-}\alpha$ immunoassay, RTA00 from R & D Systems, Abingdon, UK) whereas NO_2 concentration was measured by the Greiss reaction [20].

2.6. White blood cell counts

The percentage of each cell type (monocytes, granulocytes and lymphocytes) was determined in blood collected from the vena cava by using an automatic full digital cell counter (Melet Schoelting Laboratories); the absolute cell counts were calculated by multiplying the percentage by the total cell count for each sample.

2.7. ED2-staining

The immuno-histological detection of ED2 was performed on tissue slices as previously described [21]. Briefly, cryostat sections ($10\ \mu\text{m}$) were fixed in acetone with formaldehyde at 4°C . After incubation with 10% BSA in buffer pH 7.4, sections were incubated with mouse anti-rat ED2 antibody (DPC, Belgium). Sections were washed, incubated in methanol with H_2O_2 in order to inhibit endogenous peroxidase activity, and then incubated with peroxidase-conjugated rabbit anti-mouse antibody (DakoCytomation, Denmark). Peroxidase activity was revealed with 3,3'-diaminobenzidine-tetrahydrochloride (DAB) (Sigma, St Louis, MO). Samples were counterstained with Mayer's Haemalun (VWR International, Belgium) and mounted with DePeX mounting medium (Agar Scientific, UK). Control sections were incubated in buffer-BSA instead of primary antibody in the first step, with conjugate in the second step, and examined for non-specific staining. Semi-quantification of Kupffer cells (hepatic ED2-positive cells) was performed under microscopic analysis with squared field on 2 different liver sections (4 fields $10\ \text{mm}^2$ per liver section) for each rat in a blinded manner. Of note, the selected field contained 3 or 4 portal space.

2.8. Myeloperoxidase activity in the liver

After air drying for 10 min, cryostat sections ($10\ \mu\text{m}$) were incubated in Tris-HCl buffer (pH 7.4) containing H_2O_2 , DAB and sucrose for 20 min at 25°C in a shaking bath. After rinsing in Tris-HCl buffer, samples were counterstained with Mayer's Haemalun, dehydrated and mounted with DePeX mounting medium [21]. Semi-quantification of peroxidase-positive cells was performed as described above for ED2-staining.

2.9. Statistical analysis

Values are presented as means \pm SEM. All statistical analysis of observed variations were performed with the SPSS statistical

Table 1
Effect of a diet enriched with laminarin on body weigh gain, total food and water intakes, body weight at sacrifice and relative organ weights

	CT	LAM
Body weight gain (g)	125 \pm 3	125 \pm 3
Total food intake (g)	449.5 \pm 12.3	431.5 \pm 6.0
Total water intake (mL)	576.9 \pm 13.0	611.5 \pm 17.9
Body weight at the end of study (g)	239 \pm 4	234 \pm 3
Relative organ weight (g/100 g body weight)		
–liver	3.56 \pm 0.13	3.88 \pm 0.08 #
–spleen	0.36 \pm 0.02	0.35 \pm 0.02
–caecum wall	0.31 \pm 0.02	0.47 \pm 0.01*

Rats were fed a control diet (CT) or a diet supplemented with 10% laminarin (LAM). Lipopolysaccharides was administrated 24 h before sacrifice. Values are means \pm SEM, $n=7$. *Significant effect of LAM treatment (* $p<0.05$ and # $p=0.07$; Student t test).

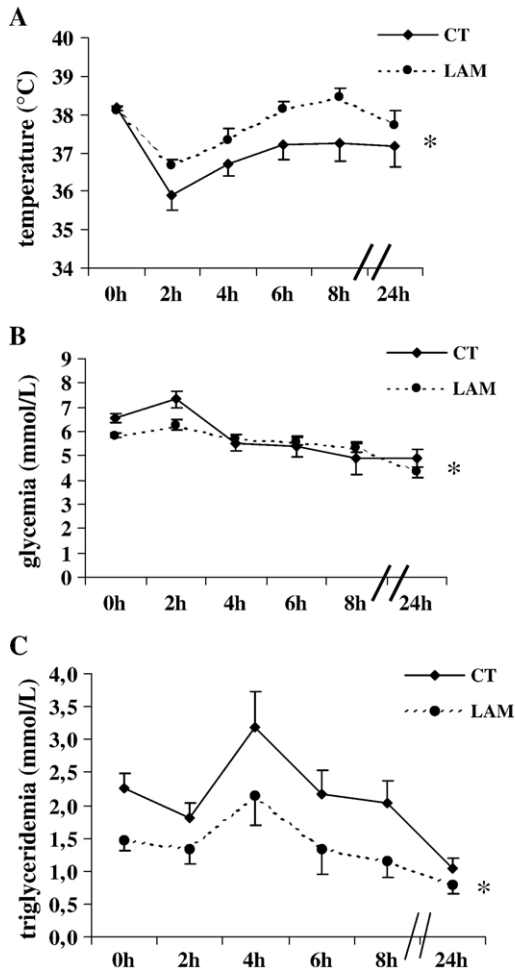


Fig. 1. Rectal temperature (A), glycemia (B) and triglyceridemia (C) after lipopolysaccharide challenge in rats fed a control diet (CT) or a diet supplemented with laminarin (LAM). Values are means±SEM, n=7. *Significant time course effect of laminarin treatment ($p < 0.05$, two-way ANOVA).

program for Windows system (SPSS, Chicago, IL). When variances were heterogeneous, logarithmic transformations of individual values were used before statistical analysis. Student *t* test was applied to compare the effect of LAM supplementation versus control diet. To compare the time course effect of LAM feeding after LPS injection on ALT, AST, LDH, body temperature, triglyceridemia and glycemia, two-way ANOVA was performed. Data were considered statistically significant at the $p < 0.05$ level.

3. Results

The supplementation of the diet with LAM during 25 days did modify neither body weight gain nor food and water intakes (Table 1). However, the weight of dehydrated stool over 24 h was higher in LAM group than in control rats (4.61 ± 0.29 g and 3.32 ± 0.17 g for LAM and CT groups, respectively, $p < 0.05$

after 12 days of treatment). Furthermore, LAM diet increased the caecal wall weight (Table 1) as well as the caecal content (3.21 ± 0.32 g and 2.15 ± 0.24 g for LAM and CT groups, respectively, $p < 0.05$). The relative liver weight 24 h after LPS administration was slightly higher in LAM-treated rats than control animals ($p = 0.07$) whereas LAM-treatment did not affect spleen weight.

3.1. Glycemia, triglyceridemia and body temperature

Rats responded to LPS injection by a transient hypothermia 2 h after injection (Fig. 1A), that was less pronounced in LAM

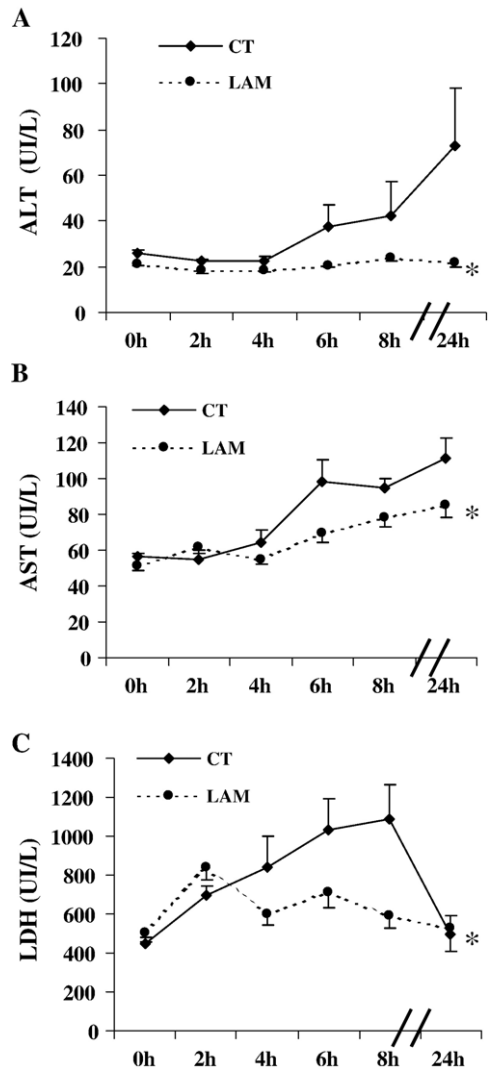


Fig. 2. Alanine aminotransferase (ALT) (A), aspartate aminotransferase (AST) (B) and lactate dehydrogenase (LDH) (C) activity in the serum after lipopolysaccharide challenge in rats fed a control diet (CT) or a diet supplemented with laminarin (LAM). Values are means±SEM, n=7. *Significant time course effect of laminarin treatment ($p < 0.05$, two-way ANOVA).

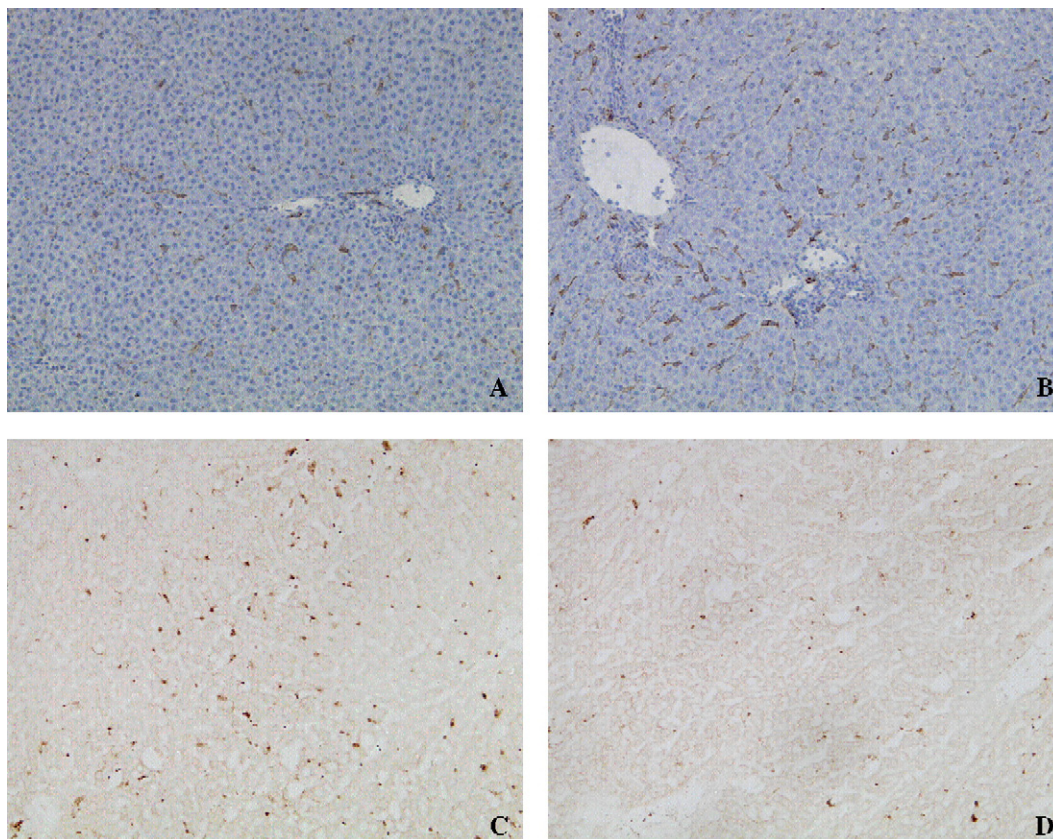


Fig. 3. Immuno-histochemical detection of ED2 (A, B) and histochemical detection of endogenous peroxidase activity (C, D) in the liver of rat 24 h after lipopolysaccharide challenge. Rats were fed a control diet (A, C) or a diet supplemented with laminarin (B, D). All ED2-positive cells are Kupffer cells. Bar=50 μ m.

rats than in controls. Post-prandial glycemia was lower in LAM-fed rats than in controls. Two hours after injection, LPS induced a transient hyperglycemia followed by hypoglycemia in control rats; the transient hyperglycemia did not occur in LAM-treated animals (Fig. 1B).

Basal triglyceridemia was lowered by LAM treatment after 3 weeks (Fig. 1C). The LPS challenge lead to an increase in triglyceridemia that peaked 4 h after LPS injection in both CT and LAM groups. However, the level of serum triglycerides remained lower in LAM group at all time points analysed.

3.2. Serum enzymes

AST, ALT and LDH activities in the serum of rats increased with time after LPS challenge (Fig. 2). However, the activity of those enzymes was significantly lower in rats previously fed with LAM.

3.3. Organ integrity and histology

We assessed organ injury by histological examination after hematoxylin-eosin staining. Marked lung congestion, oedema, alveolar septal thickening and influx of inflammatory cells

were observed 24 h after LPS administration. Moreover splenic red pulp was strongly congested whereas necrotic foci with caryorectic cells appeared in white pulp. LAM treatment did not modify these histological alterations in lung or spleen

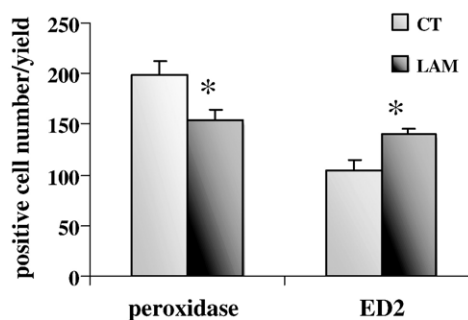


Fig. 4. Quantification of ED2-positive cells and peroxidase-positive cells in liver sections of rats fed a control diet (CT) or a diet supplemented with laminarin (LAM) after lipopolysaccharide challenge. See Materials and methods for yield definition. Values are means \pm SEM, $n=7$. *Significant effect of laminarin treatment ($p < 0.05$, student t test).

(data not shown). LPS caused also leukocyte infiltration in the liver (see below) but no necrotic foci were observed in the liver tissue in both experimental groups (data not shown).

3.4. Histological analysis of Kupffer cells and peroxidase-positive cell accumulation in the liver

ED2-staining was performed in the liver in order to detect membrane antigen of fixed tissue macrophages, namely Kupffer cells (Fig. 3A and B). The distribution of ED2-staining macrophages after LPS challenge was changed, showing a dense pattern in animals having received the supplementation of LAM as compared to control rats. Quantitative analysis of ED2-positive cells, *i.e.* Kupffer cells, confirmed this effect (Fig. 4). The detection of peroxidase activity in the liver tissue was also performed in order to assess leukocytes accumulation after LPS challenge. Peroxidase-positive cell accumulation in the liver after LPS challenge was lessened by the LAM-enriched diet as compared to control diet (Fig. 3C and D). Quantitative analysis revealed that the number of peroxidase-positive cells/yield (see Materials and methods for yield definition) was significantly lower after a diet supplemented with LAM as compared to control rats (Fig. 4).

3.5. White blood cell counts

Table 2 shows the relative proportions of the different types of white blood cells 24 h after LPS administration. The diet enriched with LAM significantly decreased the abundance of monocytes (13%) in the blood whereas no significant difference was observed for lymphocytes and granulocytes.

3.6. TNF- α , PGE₂ and NO₂ analysis in the serum

LPS-induced increase of TNF- α concentration in the serum 2 h after its administration was lower in rats previously treated with LAM than in control group (Table 3). LAM treatment also decreased PGE₂ concentration in the serum but this effect was significant only 24 h after LPS challenge. Furthermore, NO₂ concentration in the serum 24 h after LPS administration was lower in rats treated with LAM (56.1 \pm 1.7 and 35.2 \pm 1.5 μ mol/L

Table 2

Effect of a diet enriched with laminarin on white blood cell counts 24 h after LPS challenge

	CT	LAM
White blood cells ($\times 1000/\text{mm}^3$):	3.59 \pm 0.14	4.02 \pm 0.28
–monocytes (%)	2.74 \pm 0.17	2.14 \pm 0.19*
–lymphocytes (%)	33.12 \pm 2.23	31.67 \pm 2.42
–granulocytes (%)	64.15 \pm 2.13	66.18 \pm 2.39

Rats were fed a control diet (CT) or a diet supplemented with 10% laminarin (LAM). Lipopolysaccharides (LPS) was administrated 24 h before sacrifice. Values are means \pm SEM, $n=7$. *Significant effect of LAM treatment (* $p < 0.05$; Student t test).

Table 3

Effect of a diet enriched with laminarin on TNF- α and PGE₂ concentration in the serum 2 h and 24 h after LPS challenge

Time	TNF- α		PGE ₂	
	CT	LAM	CT	LAM
2 h	8043 \pm 3198	2614 \pm 1400*	2624 \pm 320	2265 \pm 397
24 h	13 \pm 3	10 \pm 2	477 \pm 37	309 \pm 22*

Rats were fed a control diet (CT) or a diet supplemented with 10% laminarin (LAM). Values are TNF- α and PGE₂ concentration in the serum (pg/mL) 2 h and 24 h after LPS challenge and represent means \pm SEM. $n=7$. *Significant effect of LAM treatment ($p < 0.05$, Student t test performed on logarithmic values). TNF- α , tumor necrosis factor- α ; PGE₂, prostaglandin E₂; LPS, lipopolysaccharide.

for CT and LAM groups, respectively; $p < 0.05$, Student t test performed on logarithmic values).

4. Discussion

In previous studies, β -glucans have been shown to enhance host defence in animal infection models as well as in humans [22–28]. The oral route seems important to get a protection by β -glucans towards infectious injury. The first observation of an effect of dietary β -glucan on systemic infection was a significant degree of protection-increased the survival rate-against anthrax in a mouse model of anthrax infection [29]. Another study suggested that β -glucan given by intra-gastric gavage to rats protects against sepsis-induced oxidative injury [28]. In the present study, we show that dietary LAM, a β -glucan from brown algae lessens acute hepatic toxicity induced by LPS, with significant improvement of serum transaminase and LDH activities. This protective effect of LAM was tissue specific, since no effect of LAM could be seen on lung or spleen morphological alterations. The decrease in ALT activity also reflects a specific protection against liver injury.

The liver is thought to be a major organ in the development of multiple organ dysfunction after sepsis [30,31]. It is known that the central cause of hepatic injury during endotoxemia is the release cytotoxic cytokines (TNF- α), reactive intermediates (NO₂) and proteolytic enzymes, namely by the accumulation and activation of neutrophils in the liver sinusoids upon LPS [8,10,14,32,33]. Indeed, in rat, an influx of neutrophils in the liver has been observed within 3–6 h after the administration of endotoxin from *E. coli* [32]. Their functions at sites of infection include phagocytosis and production of toxic compounds which facilitate the elimination of endotoxin but can also participate in severe tissue damage [8]. Kupffer cells play a major role in removal of LPS from the bloodstream [10]. Several

studies suggest that apoptosis and ingestion of neutrophils by Kupffer cells abrogate hepatic injury that may otherwise result from the uncontrolled release of such factors (for review, see Ref. [8]). In fact, we show in the present study that LAM treatment increases the number of Kupffer cells in the liver, assessed by ED2-staining, in LPS-treated animals. Interestingly, besides the increase in Kupffer cells, LAM treatment also decreased the number of myeloperoxidase-positive cells, signing a lower number of leukocytes in the liver tissue. Other authors have shown that β -glucan, given by the oral route, inhibits sepsis-activated myeloperoxidase activity [28]. Our results suggest that a higher number of Kupffer cells can enhance liver protection through both their own capacity to eliminate endotoxin and their ability to regulate the pro-inflammatory response and the anti-microbial activities of other immune cell types (neutrophils for instance) that immigrate rapidly in response to infection.

Cytokine production by monocytes plays a key role in the pathogenesis of endotoxemia. In fact, monocytes isolated from soluble β -glucan-fed mice had a lower TNF- α production upon LPS stimulation [3]. Here, we confirm that the treatment with LAM significantly lowers serum TNF- α concentration. The diet enriched with LAM has been shown to decrease monocyte number in blood of rats; a phenomenon that could explain also the decrease in serum TNF- α level. A protective effect of β -glucan linked to the lower serum TNF- α has already been described by Sener et al. in a rat model of sepsis induced by caecal ligation and puncture [28]. This would suggest that the protection offered by dietary LAM against LPS-induced tissue injury is related to a lower TNF- α production. This idea is supported by studies by Barton and Jackson [34], who found that pre-treatment with antibody to TNF- α protected mice from LPS-induced endotoxic shock. The injection of LPS to mice activated interleukin (IL)-12 and IL-18 production by Kupffer cells. As a consequence, the activation of natural killer (NK) cell release interferon gamma (IFN- γ) [33]. Therefore, IL-12, IL-18 and IFN- γ , which are representative pro-inflammatory Th1 cytokines, may also play important role in the host defence against various bacterial infections. A full study could be devoted to analyse the capability to produce the pro-inflammatory mediators cited, in cultured monocytes or Kupffer cells isolated from LAM-treated animals. NO₂ release by activated macrophages may contribute to inflammation and hepatotoxicity of endotoxin [14,35]. Interestingly, in our study, serum NO₂ level remained lower 24 h after LPS challenge in rats given LAM.

In a previous study, we have shown that higher PGE₂ release may be involved in the hepato-protection offered by treatment with non-digestible/fermentable oligosaccharides [6]. Consistent with this hypothesis, studies reported that *in vivo* administration of PGE₂ significantly protected mice or rats against liver injury and mortality after infection [22,36]. However, in the present study, PGE₂ does not seem implicated in the protection offered by LAM diet since its secretion was lower 24 h after LPS challenge in LAM-treated rat than in control animals.

LAM modules LPS-induced metabolic disturbances. The glycemic profile after LPS challenge, observed in the present study, is in accordance with the one described by Bosch et al. [37]. LPS caused a transient hyperglycemia – partly due to an increased glycogenolysis in the liver [38] – followed by a progressive decrease in glycemia, resulting from glucose utilization by peripheral tissue, and mostly by immune cells [37,39]. The lower glycemia following LPS in LAM-treated rats could be considered as protection of the liver tissue towards the catabolic effect of LPS.

The release of cytokines, which is modified by LAM, is clearly implicated in several in the metabolic response towards LPS. TNF- α provides the cryogenic signals that are involved in the maintenance of hypothermia occurring in response to LPS [6,40]. The lower TNF- α level observed in LAM animals within 24 h after LPS challenge was accompanied by the partial restoration of body temperature.

Furthermore, a close interplay also exists between lipid metabolism and infection. LPS elicits dramatic responses in the host including elevated plasma lipids levels due to the increased hepatic synthesis and secretion of triglyceride-rich lipoproteins (VLDL), and their lower catabolism by lipoprotein lipase. TNF- α -induced hypertriglyceridemia, clinically termed the “lipemia of sepsis”, was customarily thought to represent the mobilization of lipid stores to fuel the host response to infection [41,42]. In our study, the increase of TNF- α level precedes the increase in triglyceridemia due to LPS. Both parameters are lowered by LAM, supporting the implication of the lower inflammatory tone in the metabolic effect of LAM.

The interactions among food components, immune function, and protection against pathogens are complex. The influence of non-digestible oligosaccharides on immunity can be attributed to the fermentation of the compounds in the caeco-colon, that promotes the development of specific type of bacteria known to have a protective barrier effect on the mucosa [43]. We

have recently shown that non-digestible carbohydrates that promote the development of *Bifidobacteria* lowers the inflammatory tone and LPS level in obese rodents (paper submitted in *Diabetologia*). In the present study, a diet enriched with 10% LAM induced fermentation in the GIT since it increased the quantity of both dehydrated stool and caecal content. Are the immunomodulatory effects of dietary LAM described in the present study, due to a direct effect of β -glucan on immune cells, or to an indirect effect through their dietary fibres properties? Both mechanisms could be implicated. No data have been published relative to the influence of LAM administration on colonic microflora composition, but in view of the large fermentation of the compounds, the modulation of the composition and/or activity of the microflora could be one factor influencing the response towards inflammatory challenge.

Nevertheless, a direct effect of LAM on systemic immune cells can not be excluded. In fact, a recent study clearly demonstrated that LAM, once ingested, is transported from the GIT to systemic circulation; GIT-derived β -glucans can produce significantly immunomodulatory effects and increases survival in mice challenged with *Staphylococcus aureus* or *Candida albicans* [44]. If direct actions of β -glucans on immune cells are involved, it could be the consequences of link on several different receptors: the complement receptor type 3 (CR3) recognising several glucans; the non-CR3 receptor expressed in monocyte/macrophages and which is specific for β -1,3-glucans; a glycosphingolipid β -1,3-glucans receptor present on neutrophils; dectin-1 identified on macrophages as a potential β -glucan receptor; and finally the toll-like receptors (TLRs) 2 and 6 that coordinate macrophage activation in response to zymosan particles [45]. Of note, data obtained by Yan et al. indicated that Kupffer cell CR3 has a primary role in the clearance of soluble β -glucan [46]. Therefore, we may not exclude that some glucans, if they reach the liver through the portal vein, could activate Kupffer cells through CR3. This hypothesis would be interesting to test, and LAM would be an interesting candidate in view of its activity on Kupffer cells, shown in the present study.

In conclusion, if beneficial effects of β -glucans against systemic infection as well as on survival after endotoxic shock have been previously described *in vivo*, this is the first study showing that LAM added in the diet modulates intra-hepatic immune cells, by promoting Kupffer cell density, and by decreasing leukocyte accumulation inside the liver in response to LPS. This has consequences on liver tissue injury, which is less important after LAM treatment. We may propose that a

lower TNF- α and NO₂ release could play a role in the hepatoprotective effect of LAM. We do not know at this stage if this modulation is due to a direct effect of LAM or to the fermentation of this compound in the gut. Further studies are needed to highlight the receptor and post-receptor mechanisms involved in the protective effects of LAM using cultures of isolated Kupffer cells or of precision-cut liver slices, a model able to study Kupffer cells/hepatocytes interactions *in vitro* [47,48]. The relevance of the fermentation of LAM in the gut for its immunomodulatory activity process would require a full study devoted to analyse: (1) which bacterial strains are able to use LAM; (2) what is the profile of fermentation byproducts; and (3) how these byproducts may reach the liver and could modulate hepatic immune cells.

Nevertheless, LAM merits consideration as a potential therapeutic agent in the oral treatment of systemic inflammatory syndrome and associated hepatic damages.

Acknowledgments

The authors thank F. Cruz and J.C. Yvin (Goëmar) for having purchased and characterized pure laminarin used in the present study, and for their useful comments concerning these products and their physiological effects.

References

- [1] Ramphal R, Ambrose PG. Extended-spectrum beta-lactamases and clinical outcomes: current data. *Clin Infect Dis* Apr 15 2006;42(Suppl 4):S164–72.
- [2] Tacconelli E. New strategies to identify patients harbouring antibiotic-resistant bacteria at hospital admission. *Clin Microbiol Infect* Feb 2006;12(2):102–9.
- [3] Soltys J, Quinn MT. Modulation of endotoxin- and enterotoxin-induced cytokine release by *in vivo* treatment with beta-(1,6)-branched beta-(1,3)-glucan. *Infect Immun* Jan 1999;67(1):244–52.
- [4] Navarro WH, Kaplan LD. AIDS-related lymphoproliferative disease. *Blood* Jan 1 2006;107(1):13–20.
- [5] Calder PC, Kew S. The immune system: a target for functional foods? *Br J Nutr* Nov 2002;88(Suppl 2):S165–77.
- [6] Neyrinck AM, Alexiou H, Delzenne NM. Kupffer cell activity is involved in the hepatoprotective effect of dietary oligofructose in rats with endotoxic shock. *J Nutr* May 2004;134(5):1124–9.
- [7] Sheth K, Bankey P. The liver as an immune organ. *Curr Opin Crit Care* Apr 2001;7(2):99–104.
- [8] Gregory SH, Wing EJ. Neutrophil–Kupffer cell interaction: a critical component of host defenses to systemic bacterial infections. *J Leukoc Biol* Aug 2002;72(2):239–48.
- [9] Mathison JC, Ulevitch RJ. The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J Immunol* Nov 1979;123(5):2133–43.

- [10] Su GL. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. *Am J Physiol Gastrointest Liver Physiol* Aug 2002;283(2):G256–65.
- [11] Callery MP, Kamei T, Mangino MJ, Flye MW. Organ interactions in sepsis. Host defense and the hepatic-pulmonary macrophage axis. *Arch Surg* Jan 1991;126(1):28–32.
- [12] Suzuki T, Takahashi T, Yamasaki A, Fujiwara T, Hirakawa M, Akagi R. Tissue-specific gene expression of heme oxygenase-1 (HO-1) and non-specific delta-aminolevulinic synthase (ALAS-N) in a rat model of septic multiple organ dysfunction syndrome. *Biochem Pharmacol* Jul 15 2000;60(2):275–83.
- [13] Decker K. Biologically active products of stimulated liver macrophages (Kupffer cells). *Eur J Biochem* Sep 11 1990;192(2):245–61.
- [14] Laskin DL, Pendino KJ. Macrophages and inflammatory mediators in tissue injury. *Annu Rev Pharmacol Toxicol* 1995;35:655–77.
- [15] Brown GD, Gordon S. Immune recognition of fungal beta-glucans. *Cell Microbiol* Apr 2005;7(4):471–9.
- [16] Deville C, Damas J, Forget P, Dandriofosse G, Peulen O. Laminarin in the dietary fibre concept. *J Sci Food Agric* Jul 2004;84(9):1030–8.
- [17] Heuff G, van der Ende MB, Boutkan H, Prevoo W, Bayon LG, Fleuren GJ, et al. Macrophage populations in different stages of induced hepatic metastases in rats: an immunohistochemical analysis. *Scand J Immunol* Jul 1993;38(1):10–6.
- [18] Anderson WA, Kang YH, Mohla S. Mammalian endogenous peroxidases as cellular markers and as biosynthetic endpoints of hormone-mediated activity: viewpoint from cytochemistry. *Prog Histochem Cytochem* 1979;11(4):1–27.
- [19] Daubioul CA, Taper HS, De Wispelaere LD, Delzenne NM. Dietary oligofructose lessens hepatic steatosis, but does not prevent hypertriglyceridemia in obese Zucker rats. *J Nutr* May 2000;130(5):1314–9.
- [20] Bishop-Bailey D, Larkin SW, Warner TD, Chen G, Mitchell JA. Characterization of the induction of nitric oxide synthase and cyclo-oxygenase in rat aorta in organ culture. *Br J Pharmacol* May 1997;121(1):125–33.
- [21] Neyrinck AM, Margagliotti S, Delzenne NM. Insight into the involvement of Kupffer cell-derived mediators in the hepatoprotective effect of glycine upon inflammation: study on rat precision-cut liver slices. *Inflamm Res* Mar 2005;54(3):106–12.
- [22] Cisneros RL, Gibson III FC, Tzianabos AO. Passive transfer of poly-(1–6)-beta-glucotriose-(1–3)-beta-glucopyranose glucan protection against lethal infection in an animal model of intra-abdominal sepsis. *Infect Immun* Jun 1996;64(6):2201–5.
- [23] Babineau TJ, Hackford A, Kenler A, Bistran B, Forse RA, Fairchild PG, et al. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-glucan) in high-risk surgical patients. *Arch Surg* Nov 1994;129(11):1204–10.
- [24] Babineau TJ, Marcello P, Swails W, Kenler A, Bistran B, Forse RA. Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-glucan) in high-risk surgical patients. *Ann Surg* Nov 1994;220(5):601–9.
- [25] Kermode DS, Gates H, Kaiser AB. Prophylactic anti-infective activity of poly-[1–6]-beta-D-glucopyranosyl-[1–3]-beta-D-glucopyranose glucan in a guinea pig model of staphylococcal wound infection. *Antimicrob Agents Chemother* Mar 1998;42(3):545–9.
- [26] Tzianabos AO, Cisneros RL. Prophylaxis with the immunomodulator PGG glucan enhances antibiotic efficacy in rats infected with antibiotic-resistant bacteria. *Ann N Y Acad Sci* Oct 25 1996;797:285–7.
- [27] Demir G, Klein HO, Mandel-Molinas N, Tuzuner N. Beta glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *Int Immunopharmacol* Jan 2007;7(1):113–6.
- [28] Sener G, Toklu H, Ercan F, Erkanli G. Protective effect of beta-glucan against oxidative organ injury in a rat model of sepsis. *Int Immunopharmacol* Aug 2005;5(9):1387–96.
- [29] Kournikakis B, Mandeville R, Brousseau P, Ostroff G. Anthrax-protective effects of yeast beta 1,3 glucans. *MedGenMed* Mar 21 2003;5(1):1.
- [30] Sheth K, Bankey P. The liver as an immune organ. *Curr Opin Crit Care* Apr 2001;7(2):99–104.
- [31] Simpson KJ, Lukacs NW, Colletti L, Strieter RM, Kunkel SL. Cytokines and the liver. *J Hepatol* Dec 1997;27(6):1120–32.
- [32] Bautista AP, Meszaros K, Bojta J, Spitzer JJ. Superoxide anion generation in the liver during the early stage of endotoxemia in rats. *J Leukoc Biol* Aug 1990;48(2):123–8.
- [33] Seki S, Habu Y, Kawamura T, Takeda K, Dobashi H, Ohkawa T, et al. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+ T cells in T helper 1 immune responses. *Immunol Rev* Apr 2000;174:35–46.
- [34] Barton BE, Jackson JV. Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. *Infect Immun* Apr 1993;61(4):1496–9.
- [35] Alexander B. The role of nitric oxide in hepatic metabolism. *Nutrition* Apr 1998;14(4):376–90.
- [36] Takano M, Nishimura H, Kimura Y, Washizu J, Mokuno Y, Nimura Y, et al. Prostaglandin E2 protects against liver injury after *Escherichia coli* infection but hampers the resolution of the infection in mice. *J Immunol* Sep 15 1998;161(6):3019–25.
- [37] Bosch MA, Garcia R, Pagani R, Portoles MT, Diaz-Laviada I, Abarca S, et al. Induction of reversible shock by *Escherichia coli* lipopolysaccharide in rats. Changes in serum and cell membrane parameters. *Br J Exp Pathol* Dec 1988;69(6):805–12.
- [38] Mandl J, Wall C, Lerant I, Falus A, Machovich R, Thurman RG. Endotoxin and fibrinogen degradation product-D have different actions on carbohydrate metabolism: role of Kupffer cells. *FEBS Lett* Nov 27 1995;376(1–2):65–6.
- [39] Spolarics Z, Spitzer JJ. Acute endotoxin tolerance is accompanied by stimulated glucose use in macrophage rich tissues. *Biochem Biophys Res Commun* Jun 6 1995;211(1):340–6.
- [40] Tollner B, Roth J, Storr B, Martin D, Voigt K, Zeisberger E. The role of tumor necrosis factor (TNF) in the febrile and metabolic responses of rats to intraperitoneal injection of a high dose of lipopolysaccharide. *Pflugers Arch* Oct 2000;440(6):925–32.
- [41] Harris HW, Gosnell JE, Kumwenda ZL. The lipemia of sepsis: triglyceride-rich lipoproteins as agents of innate immunity. *J Endotoxin Res* 2000;6(6):421–30.
- [42] Berbee JF, Havekes LM, Rensen PC. Apolipoproteins modulate the inflammatory response to lipopolysaccharide. *J Endotoxin Res* 2005;11(2):97–103.
- [43] Buddington RK, Kelly-Quagliana K, Buddington KK, Kimura Y. Non-digestible oligosaccharides and defense functions: lessons learned from animal models. *Br J Nutr* May 2002;87(Suppl 2):S231–9.
- [44] Rice PJ, Adams EL, Ozment-Skelton T, Gonzalez AJ, Goldman MP, Lockhart BE, et al. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *J Pharmacol Exp Ther* Sep 2005;314(3):1079–86.

- [45] Engstad CS, Engstad RE, Olsen JO, Osterud B. The effect of soluble beta-1,3-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. *Int Immunopharmacol* Oct 2002;2(11):1585–97.
- [46] Yan J, Vetvicka V, Xia Y, Hanikyrova M, Mayadas TN, Ross GD. Critical role of Kupffer cell CR3 (CD11b/CD18) in the clearance of IgM-opsonized erythrocytes or soluble beta-glucan. *Immunopharmacology* Jan 2000;46(1):39–54.
- [47] Neyrinck AM, Gomez C, Delzenne NM. Precision-cut liver slices in culture as a tool to assess the physiological involvement of Kupffer cells in hepatic metabolism. *Comp Hepatol* Jan 14 2004;3(Suppl 1):S45.
- [48] Neyrinck A, Eeckhoudt SL, Meunier CJ, Pampfer S, Taper HS, Verbeeck RK, et al. Modulation of paracetamol metabolism by Kupffer cells: a study on rat liver slices. *Life Sci* 1999;65(26):2851–9.